



UNIVERSITI PUTRA MALAYSIA

**MOLECULAR AND IMMUNOLOGICAL IDENTIFICATIONS OF
GIARDIA SP. ISOLATED FROM HUMANS, DOGS AND RODENTS**

NGAH ZASMY UNYAH

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OF *GIARDIA* SP. ISOLATED FROM HUMANS,
DOGS AND RODENTS**

By

NGAH ZASMY UNYAH

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia
In Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

October 2004



DEDICATION

This work is dedicated to my family and friends.
Thank you for your support, love and friendship.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

**MOLECULAR AND IMMUNOLOGICAL IDENTIFICATIONS
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October 2004

Chairman : Professor Hj. Wan Omar Abdullah, Ph.D.

Faculty : Medicine and Health Sciences

Concentrations and staining methods for identification of *Giardia* parasites in faecal materials from humans and animals are still the routine methods of diagnosis of giardiasis. Introduction of new and sensitive immunological and molecular methods will definitely facilitate diagnosis and the identification of various *Giardia* species that will ultimately improve clinical management and control of disease transmission. The identification of specific proteins of *Giardia* parasites, which are genus and species specific, may further improve the specificity and sensitivity of diagnostic methods. In this study, identification and confirmation of the species of *Giardia* parasite found in Malaysia, particularly in humans, dogs and rodents were done by Polymerase Chain Reaction (PCR) using species-specific primers. Specific Heat-Shock Protein (HSP) as markers for species identification were done using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western Immunoblotting (WI). These markers were used as antigens for the

production of species-specific monoclonal antibodies (MAb). The objectives of this study were: i) to identify the common *Giardia* sp. infecting humans and other mammals (dogs and rodents) in Malaysia using specific *Giardia* primers by PCR; ii) to produce *in vitro* and detect immunogenic HSP using polyclonal sera from rabbit immunised with antigens from known *Giardia* species on WI, and iii) to produce species-specific MAb from the identified HSP markers. Microscopically, this study has observed that *G. intestinalis* (GI) and *G. duodenalis* (GD) were indistinguishable but *G. muris* (GM) can be distinguished from GI or GD. In addition, this study has also confirmed that PCR using species-specific primers was more reliable and accurate in detecting the variant of GI found in humans and dogs. GD isolates recovered from dogs was found to be the actual variant of GI of humans. Clear morphological differentiations and identifications of GM and GI based on microscopical examination were observed and similar results were obtained by PCR using species-specific primers of respective species of *Giardia*. However, the SDS-PAGE and WI failed to identify species-specific HSP markers, but WI using immunised rabbit sera detected four immunogenic HSP, with the molecular weight of 30 kDa, 34 kDa, 58 kDa and 66 kDa. These four immunogenic HSP were detected at 25 °C, 37 °C and 50 °C in both GI and GM. Three species-specific MAbs were produced using the combinations of the four immunogenic HSPs as antigens. These MAbs were designated as (i) [32 kDa HSPMAbGi(IgG3)], (ii) [29 kDa HSPMAbGm(IgM)], and (iii) [20 kDa HSPMAbGi(IgG1)]. [32 kDa HSPMAbGi(IgG3)] MAb was specific for GI variant found in humans, [29 kDa HSPMAbGm(IgM)] MAb was specific for GM and [20 kDa HSPMAbGi(IgG1)] was specific for GI variant in both humans and dogs. These findings suggest that GI is the main causative agent of giardiasis in both humans and urban dogs in Malaysia.

GM is the main *Giardia* parasite infecting rodents in both rural and urban areas in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENGenalPASTIAN MOLEKUL DAN IMUNOLOGI TERHADAP
GIARDIA SP. YANG DIPENCILKAN DARI MANUSIA,
ANJING DAN RODEN**

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Kaedah pewarnaan dan konsentration feses bagi pengenalpastian *Giardia* yang terdapat dalam feses manusia dan haiwan adalah kaedah-kaedah yang masih lagi penting dan digunakan secara rutin dalam diagnosis giardiasis. Kaedah-kaedah terkini imunologi dan molekul dalam diagnosis akan meningkatkan lagi penambahbaikan diagnosis yang akhirnya akan membantu dalam pengurusan klinikal dan kawalan penularan infeksi. Pengenalpastian protein-protein yang spesifik untuk genus and spesies *Giardia* akan dapat meningkatkan lagi kespesifikan pengenalpastian parasit ini. Dalam kajian ini, pengenalpastian spesies *Giardia* yang terdapat di Malaysia, terutamanya yang menjangkiti manusia, anjing dan rodent telah dilakukan melalui kaedah Tindakbalas Berangkai Polimeras (PCR) dengan menggunakan primer yang spesifik untuk spesies *Giardia*. Profil Protein Kejutan Haba (HSP) untuk masing-masing spesies *Giardia* telah dikenalpasti melalui kaedah “Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis” (SDS-PAGE) dan “Western Immunoblotting” (WI). Tidak ada perbezaan profil protein di antara



ketiga-tiga spesies *Giardia* ini namun terdapat perbezaan antiserum poliklonal arnab yang bertindakbalas secara spesifik dengan HSP untuk masing-masing spesies *Giardia*. HSP juga telah digunakan sebagai antigen bagi penghasilan antibodi monoklonal (MAb) yang spesifik untuk spesies *Giardia*. Objektif kajian ini ialah :

- i) mengenalpasti spesies *Giardia* yang menjangkiti manusia dan haiwan mamalia (anjing dan rodent) yang terdapat di Malaysia dengan menggunakan primer yang spesifik bagi spesies untuk *Giardia* dalam kaedah PCR, ii) menghasilkan dan mengesan HSP yang imunogenik sebagai petanda yang spesifik untuk spesies *Giardia* melalui penggunaan serum poliklonal arnab yang telah diimmunisasi dengan antigen spesies *Giardia* yang mana pengenalpastian protein ini adalah melalui kaedah WI, dan iii) menghasilkan MAb yang spesifik untuk spesies *Giardia* menggunakan HSP yang telah dikenalpasti sebagai antigen. Hasil penyelidikan mendapati, kaedah PCR menggunakan primer spesifik untuk spesies dengan tepatnya dapat mengesan dan membezakan di antara varian spesies *G. intestinalis* (GI) yang terdapat dalam manusia dan anjing. Isolat *G. duodenalis* (GD) yang dijumpai pada anjing adalah merupakan salah satu varian GI, sama seperti yang terdapat pada manusia. Perbezaan dan pengenalpastian morfologi dapat dilihat dengan jelas di antara *G. muris* (GM) dan GI, hasil kajian ini adalah sama seperti hasil kajian yang di kesan oleh PCR dengan menggunakan spesifik untuk spesies primer khas untuk GM dan GI. Keputusan penyelidikan ini mendapati, GI adalah agen penyebab utama penyakit giardiasis pada manusia dan anjing terutama di kawasan bandar di Malaysia. GM pula adalah parasit yang menjangkiti khususnya pada rodent di kedua-dua kawasan bandar dan luar bandar di Malaysia. Kaedah SDS-PAGE dan WI didapati tidak dapat mengenalpasti kehadiran spesifik untuk spesies protein HSP sebagai petanda, tetapi kaedah WI dengan menggunakan serum arnab

yang telah diimunisasikan, dapat mengenalpasti empat imunogenik protein HSP yang mempunyai berat molekul 30 kDa, 34 kDa, 58 kDa dan 66 kDa. Keempat-empat HSP ini didapati dihasilkan pada suhu 25 °C, 37 °C dan 50 °C oleh GI dan juga GM. Keempat-empat HSP ini merupakan calon yang sesuai digunakan sebagai antigen dalam penghasilan MAb. Tiga MAb yang spesifik kepada spesies *Giardia* telah berjaya dihasilkan dengan menggunakan gabungan keempat-empat jenis imunogenik protein HSP sebagai antigen. Tiga MAb yang telah dihasilkan adalah (i) [32 kDa HSPMAbGi(IgG3)], (ii) [29 kDa HSPMAbGm(IgM)], dan (iii) [20 kDa HSPMAbGi(IgG1)]. MAb [32 kDa HSPMAbGi (IgG3)] didapati spesifik hanya terhadap varian GI yang terdapat pada manusia, MAb [29 kDa HSPMAbGm(IgM)] hanya spesifik kepada GM dan, [20 kDa HSPMAbGi(IgG1)] didapati spesifik kepada kedua-dua varian GI yang terdapat pada manusia dan anjing.

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I certify that an Examination Committee met on 29th October 2004 to conduct the final examination of Ngah Zasmay Unyah on his Doctor of Philosophy thesis entitled "Molecular and Immunological Identification of *Giardia* sp. Isolated from Humans, Dogs and Rodents" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my own original work except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.


NGAH ZASMY UNYAH

Date: 26 November 2003

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